Towards an understanding of the side effects of anti-HIV drugs using Caenorhabditis elegans

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Citation for published version (APA):
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The research described in this thesis was carried out in the group of Molecular Biology and Microbial Food Safety at the Swammerdam Institute of Life Sciences, Faculty of Science, University of Amsterdam, The Netherlands.

The research was funded by MacGillavry fellowship at the University of Amsterdam. The financial support of the Hercules Foundation (AUGE/013) and the Belgian Research Fund (B/11599/17) are gratefully acknowledged.

The publication of this doctoral thesis received financial assistance from the University of Amsterdam.

Cover: An adaptation by the author of the ‘Flammarion engraving’; a wood engraving by an unknown artist, so named because its first documented appearance is in Camille Flammarion’s 1888 book L’atmosphère: météorologie populaire (“The Atmosphere: Popular Meteorology”). It has been used to represent a supposedly medieval cosmology, including a flat earth bounded by a solid and opaque sky, or firmament, and also as a metaphorical illustration of either the scientific or the mystical quests for knowledge.
Towards an understanding of the side effects of anti-HIV drugs using *Caenorhabditis elegans*

**ACADEMISCH PROEFSCHRIFT**

ter verkrijging van de graad van doctor
aan de Universiteit van Amsterdam
op gezag van de Rector Magnificus
prof. dr. D. C. van den Boom
ten overstaan van een door het College voor Promoties ingestelde
commissie, in het openbaar te verdedigen in de Agnietenkapel
op vrijdag 12 februari 2016, te 14:00 uur

door

**Reuben Luke Smith**

geboren te Taunton, Verenigd Koninkrijk
Promotiecommissie:

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              prof. dr. B.P. Braeckman. University of Ghent
              prof. dr. W.H. De Vos. University of Antwerp

Faculteit der Natuurwetenschappen, Wiskunde en Informatica
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Abbreviations

$\Delta \Psi_{mt}$  Mitochondrial membrane potential

2DNAGE  two-dimensional neutral/neutral agarose gel-electrophoresis

ADP  Adenosine 5’-diphosphate

AIDS  Acquired immunodeficiency syndrome

ALCAR  Acetyl-L-carnitine

AOX  Anti-oxidant

ApoB  Apolipoprotein B

ART  Antiretroviral therapy

ATP  Adenosine 5’-triphosphate

AZT  Zidovudine

CD4$^+$  Cluster of differentiation-4 positive cells

C. elegans  Caenorhabditis elegans

Complex I  NADH dehydrogenase

Complex II  Succinate dehydrogenase

Complex III  CoQH$_2$-cytochrome c reductase

Complex IV  Cytochrome c oxidase

Complex V  $F_0F_1$-ATPase

CoQ  Coenzyme Q

COX  Cytochrome c oxidase

CYP  Cytochrome P450

d4T  Stavudine

ddC  Zalcitabine

ddi  Didanosine

ddNTP  Dideoxynucleoside analogue triphosphates

DEG  (Statistically) differentially expressed gene

dH$_2$O  Distilled water

DMSO  Dimethylsulfoxide

DNA  Deoxyribonucleic acid

DNC  Deoxynucleotide carrier

dNTP  Deoxy-nucleotidetriphosphate
<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP</td>
<td>Diphosphate</td>
</tr>
<tr>
<td>E. coli</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>EI</td>
<td>Entry inhibitor</td>
</tr>
<tr>
<td>ENT</td>
<td>Equilibrative nucleoside transporter</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic reticulum</td>
</tr>
<tr>
<td>FAD(H)</td>
<td>Flavin adenine dinucleotide</td>
</tr>
<tr>
<td>FDA</td>
<td>U.S. food and drug administration</td>
</tr>
<tr>
<td>FLT</td>
<td>Alovudine</td>
</tr>
<tr>
<td>FUdR</td>
<td>5-Fluoro-2'-deoxyuridine</td>
</tr>
<tr>
<td>GFP</td>
<td>Green fluorescent protein</td>
</tr>
<tr>
<td>GO</td>
<td>Gene ontology</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>GST</td>
<td>Glutathione-S-transferase</td>
</tr>
<tr>
<td>H$_2$DCFDA</td>
<td>2',7'-Dichlorofluorescein</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>HAART</td>
<td>Highly active antiretroviral therapy</td>
</tr>
<tr>
<td>HEK293T</td>
<td>Human Embryonic Kidney 293 cell line</td>
</tr>
<tr>
<td>HepG2</td>
<td>Human liver carcinoma cell line</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>IDV</td>
<td>Indinavir</td>
</tr>
<tr>
<td>II</td>
<td>Integrase inhibitor</td>
</tr>
<tr>
<td>KEGG</td>
<td>Kyoto Encyclopedia of Genes and Genomes</td>
</tr>
<tr>
<td>L1</td>
<td>Nematode larval stage 1</td>
</tr>
<tr>
<td>L4</td>
<td>Nematode larval stage 4</td>
</tr>
<tr>
<td>LAA</td>
<td>L-ascorbic Acid</td>
</tr>
<tr>
<td>M9</td>
<td>Buffer for <em>C. elegans</em></td>
</tr>
<tr>
<td>MI</td>
<td>Maturation inhibitor</td>
</tr>
<tr>
<td>MP</td>
<td>Monophosphate</td>
</tr>
<tr>
<td>MRC</td>
<td>Mitochondrial respiratory chain (a.k.a. electron transport chain)</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>MRP</td>
<td>Multidrug resistance-related protein</td>
</tr>
</tbody>
</table>
Abbreviations

mtDNA  Mitochondrial DNA
NAC  N-acetyl cysteine
NAD(H)  Nicotinamide adenine dinucleotide
nDNA  Nuclear DNA
NFV  Nelfinavir
NGM  Nematode growth medium
NNRTI  Non-nucleoside reverse transcriptase inhibitor
NRTI  Nucleoside reverse transcriptase inhibitor
OCR  Oxygen consumption rate
PBMC  Human peripheral blood mononuclear cells
PCA  Principal component analysis
PCR  Polymerase chain reaction
PI  Protease inhibitor
PQ  Paraquat
RNAi  RNA interference
RNAseq  RNA sequencing
ROS  Reactive oxygen species; In this thesis ROS is taken to encompass the initial species generated by reduction of oxygen (superoxide or hydrogen peroxide) as well as their secondary reactive products
RTV  Ritonavir
S.E.M.  Standard error of the mean
SIV  Simian immunodeficiency virus
SOD  Superoxide dismutase
SQV  Saquinavir
TK  Thymidine kinase
TP  Triphosphate
Trolox  6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid (α-tocopherol derivative)
UPRx  Endoplasmic reticulum unfolded protein response
UPRmt  Mitochondrial unfolded protein response
UPS  Ubiquitin proteasome system